138.(New) The kit of Claim 135, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide is operatively linked to the vector.

139.(New) The kit of Claim 135, wherein the transfecting agent comprises a lipid transfecting agent.

140.(New) The kit of Claim 135, wherein the transfecting agent further comprises a c-kit ligand.

141.(New) The kit of Claim 135, further comprising an immunosuppressing agent.

142.(New) The kit of Claim 141, wherein the immunosuppressing agent is selected from the group consisting of cyclosporin and corticosteroids.

143.(New) The kit of Claim 135, wherein the genetic selection marker comprises a gene expressing a detectable product driven by a spermatogonia-specific promoter.

144.(New) The kit of Claim 135, wherein said promoter is selected from the group consisting of c-kit promoter, b-Myb promoter, c-raf-1 promoter, ATM (ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, and FRMI (from fragile X site) promoter.

145. The kit of Claim 135, wherein at least one polynucleotide comprises at least one polynucleotide sequence encoding a genetic selection marker.---.

REMARKS

Applicant's Preliminary Amendment is submitted together with a divisional application directed to the subject matter of Claims 114-126, as originally filed in pending parent U.S. Serial No. 09/191,920, which claim was designated Group V in a restriction requirement, mailed March 24, 2000.

The amendment of the title (at page 1, lines 1-3), is to bring these into conformity with the new Claims 135-145.

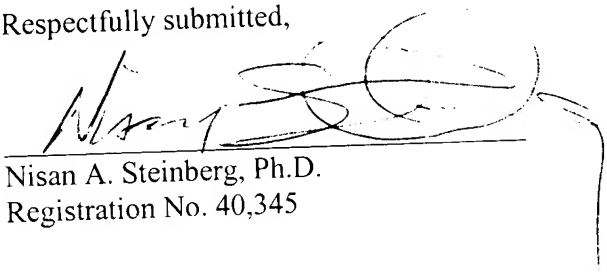
Applicant believes that no new matter is introduced by any amendments made herein.

At page 1, line 4, Applicant has added continuing data explaining the relationship to U.S. Serial No. 09/191,920 and other divisions and continuations thereof.

Applicant's cancellation of Claims 1-134 is made without prejudice. New Claim 135 is added. Support is found, e.g., in Claims 114-126, as originally filed.

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney at (213) 896-6665.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

In the Title, at page 1, lines 1-3, please delete the entire title, and insert therefor:

--KIT FOR TRANSFECTION, STORAGE AND TRANSFER OF MALE GERM CELLS FOR GENERATION OF TRANSGENIC SPECIES [& GENETIC THERAPIES]--.

At page 1, line 4, please delete the entire one-sentence paragraph, and insert the following:

--This application is a division of U.S. Non-provisional Application No. 09/191,920, filed on November 13, 1998, which claims the benefit of U.S. Provisional Application No. 60/065825, filed on November 14, 1997. This application is also related to U.S. Serial No._____, filed on November 12, 2001, U.S. Serial No._____, filed on November 12, 2001, and U.S. Serial No._____, filed on November 12, 2001, which are all divisions of U.S. Serial No. 09/191,920. This application is also related to U.S. Serial No. 09/272,443, filed March 19, 1999, which is a continuation of 09/191,920.--.

At page 4, line 14 through page 15, line 1, please delete the entire paragraph, and insert therefor the following:

--This invention also relates to a novel method for the isolation of spermatogonia, comprising obtaining spermatogonia from a mixed population of testicular cells by extruding the cells from the seminiferous tubules and gentle enzymatic disaggregation. The spermatogonia or stem cells which are to be genetically modified, may be isolated from a mixed cell population by a novel method including the utilization of a promoter sequence, which is only active in cycling spermatogonia stem cell populations, for example, b-Myb or a spermotogonia specific promoter, such as the c-kit promoter region, c-raf-1 promoter, ATM ([ataxaia]ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, or FRMI (from fragile X site) promoter, optionally linked to a reporter construct, for example, the Green Fluorescent Protein Gene (EGFP). These unique promoter sequences drive the expression of the reporter construct only in the cycling spermatogonia. The spermatogonia, thus, are the only cells in the mixed population which will express the reporter construct and they, thus, may be isolated on this basis. In the case of the green

fluorescent reporter construct, the cells may be sorted with the aid of, for example, a FACs scanner set at the appropriate wavelength or they may be selected by chemical methods.--.

At page 10, lines 11-17, please delete the entire paragraph and insert therefor the following:

--"Gene delivery (or transfection) mixture", in the context of this patent, means selected genetic material together with an appropriate vector mixed, for example, with an effective amount of lipid transfect[ion]ing agent. The amount of each component of the mixture is chosen so that the transfection of a specific species of germ cell is optimized. Such optimization requires no more than routine experimentation. The ratio of DNA to lipid is broad, preferably about 1: 1, although other proportions may also be utilized depending on the type of lipid agent and the DNA utilized. This proportion is not crucial.--.

At page 20, lines 15-22, please delete the entire paragraph and insert therefor the following:

--The GFP DNA-transferrin-polylysine viral complexes, prepared as described in Example 4 above, were delivered into the seminiferous tubules of three (3)-week-old B6D2F1 male mice. The DNA delivery by transferrin receptor-mediated endocytosis is described by Schmidt et al. and Wagner et al. (Schmidt et al., Cell 4: 41-51 (1986); Wagner, E., et al. PNAS (1990), (USA) 81: 3410-3414 (1990)). In addition, this delivery system relies on the capacity of adenoviruses to disrupt cell vesicles, such as endosomes and release the contents entrapped therein. The transfection efficiency of this system is almost 2,000 fold higher than lipofection.--.

IN THE CLAIMS:

Please cancel Claims 1-134, without prejudice, as originally filed with parent application 09/191,920, and add the following new Claims 135-145 as being directed to the subject matter of designated claim Group V, which is herein elected.

--135.(New) A kit for the transfection of a male non-human vertebrate's germ cells, comprising at least one transfecting agent, and at least one polynucleotide encoding a gene product in operable linkage with a promoter, in the presence of a gene delivery mixture comprising at least one transfecting agent, and optionally a polynucleotide encoding a genetic selection marker; and instructions for using said kit to transfect a male non-human vertebrate's germ cells.

136.(New) The kit of Claim 135, wherein the transfecting agent is selected from the group consisting of liposomes, viral vectors, transferrin-polylysine enhanced viral vectors, retroviral vectors, lentiviral vectors, and uptake enhancing DNA segments, or comprises a mixture of any members of said group.

137.(New) The kit of Claim 135, wherein the transfecting agent comprises a viral vector selected from the group consisting of retroviral vectors, adenoviral vectors, transferrin-polylysine enhanced adenoviral vectors, human immunodeficiency virus vectors, lentiviral vectors, Moloney murine leukemia virus-derived vectors, mumps vectors, DNAs that facilitate polynucleotide uptake by and release into the cytoplasm of germ cells, or comprises an operative fragment of- or mixture of any members of said group.

138.(New) The kit of Claim 135, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide is operatively linked to the vector.

139.(New) The kit of Claim 135, wherein the transfecting agent comprises a lipid transfecting agent.

140.(New) The kit of Claim 135, wherein the transfecting agent further comprises a c-kit ligand.

141.(New) The kit of Claim 135, further comprising an immunosuppressing agent.

142.(New) The kit of Claim 141, wherein the immunosuppressing agent is selected from the group consisting of cyclosporin and corticosteroids.

143.(New) The kit of Claim 135, wherein the genetic selection marker comprises a gene expressing a detectable product driven by a spermatogonia-specific promoter.

144.(New) The kit of Claim 135, wherein said promoter is selected from the group consisting of c-kit promoter, b-Myb promoter, c-raf-1 promoter, ATM (ataxia-telangiectasia) promoter, RBM (ribosome binding motif) promoter, DAZ (deleted in azoospermia) promoter, XRCC-1 promoter, HSP 90 (heat shock gene) promoter, and FRMI (from fragile X site) promoter.

145. The kit of Claim 135, wherein at least one polynucleotide comprises at least one polynucleotide sequence encoding a genetic selection marker.--.